^{1}H-^{15}N CIGAR – Optimizing for Long-Range Nitrogen Detection

Application Note

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Abstract

VnmrJ 3 software provides easy-to-use, interactive tools for setting up advanced experiments. This allows even novice users to get critical information about their research samples using the most advanced NMR experiments available. This application note is just one of a series designed to provide step-by-step guidance for setting up sophisticated experiments to collect exactly the data you need for your analyses.

Introduction

^{1}H-^{15}N long-range data can be an extremely valuable tool for solving small-molecule structure elucidation problems. While the HMBC experiment (heteronuclear multiple-bond correlation) has long been the standard pulse sequence used for these kinds of studies, this family of pulse sequences has the limitation of being optimized for a single long-range coupling constant during evolution. For ^{1}H-^{13}C spin systems this is usually not a significant issue as the coupling constants are well known and tend to cluster around specific values. However, steric crowding caused by the lone pair of electrons on a nitrogen atom can skew the bond angles around this atom and thereby shift the long-range coupling constants across a broad range of values. As a result, the operator's selection of an appropriate ^{15}N-HMBC evolution period for a given sample is often just a guess.

To address this issue, Hadden *et al.*[2] published a paper describing the CIGAR-HMBC experiment (constant time inverse-detected gradient accordion rescaled heteronuclear multiple-bond correlation). This pulse sequence uses an accordion-based constant evolution period to sample long-range coupling constants across a range of values. The CIGAR family of experiments was first developed to allow easier detection of small coupling constants in ^{1}H-^{13}C systems, but it conveniently overcomes the limitation of having a single optimized coupling evolution associated with HMBC. As such, this approach is very well suited to the collection of ^{1}H-^{15}N long-range survey spectra.

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A CIGAR Example

As an illustration of using the CIGAR experiment to improve the quality of ^{1}H-^{15}N long range NMR data, data sets were collected on a sample of brucine (see Figure 1) in $CDCl_3$. As shown in Figure 2, the average response intensity observed in the CIGAR experiment is superior to that obtained by the HMBC. This may not be the case for a specific correlation where J_{CH} matches the coupling constant used to calculate the evolution period for HMBC, but the overall improvement in average signal intensity is well worth this compromise.

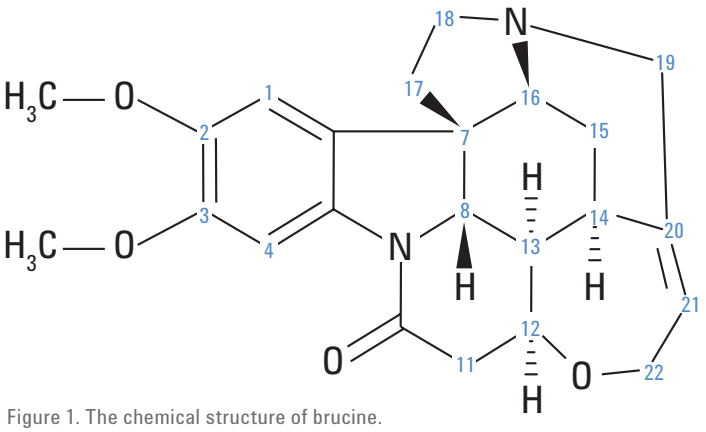

Figure 1. The chemical structure of brucine.

Experimental Method

The CIGAR experiment is easy to set up in VnmrJ 3 using the following steps:

1. Select the **CIGAR** or **CIGARAD** protocol from the Experiment Selector (the CIGARAD sequence will provide better excitation over the wide spectral width required in the ^{15}N dimension and is the preferred method). The experiment can be run as a new study, a continuation of an existing study, or in foreground (Figure 3).

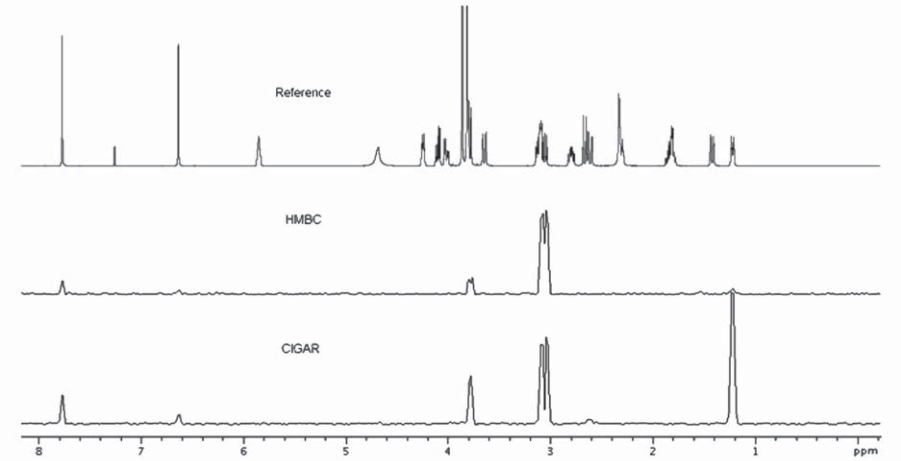

Figure 2. Comparison of HMBC and CIGAR response intensity at the frequency of the amide nitrogen resonance (F1 traces). Note the better intensity observed in the CIGAR for a broad range of responses.

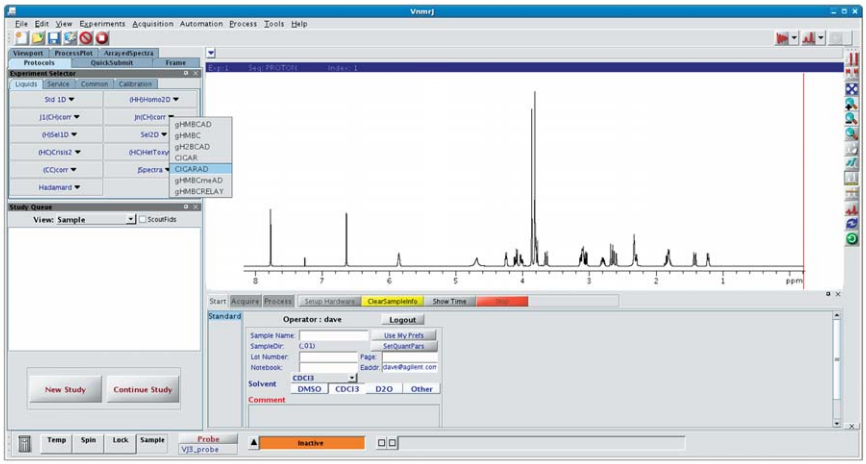

Figure 3. Setting up the CIGAR pulse sequence, step 1.

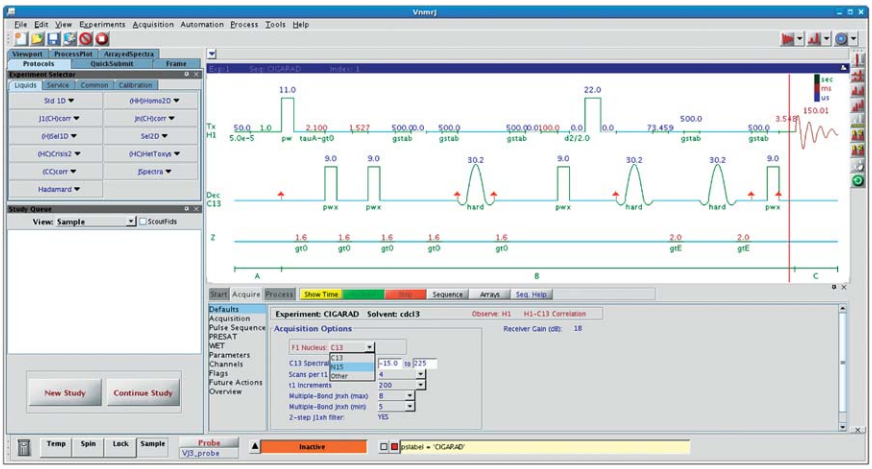

Figure 4. Setting up the CIGAR pulse sequence, step 2. By default, the experiment is configured for ^{1}H-^{13}C acquisition. Select **N15** from the F1 Nucleus pull-down to convert the parameter set to ^{1}H-^{15}N.

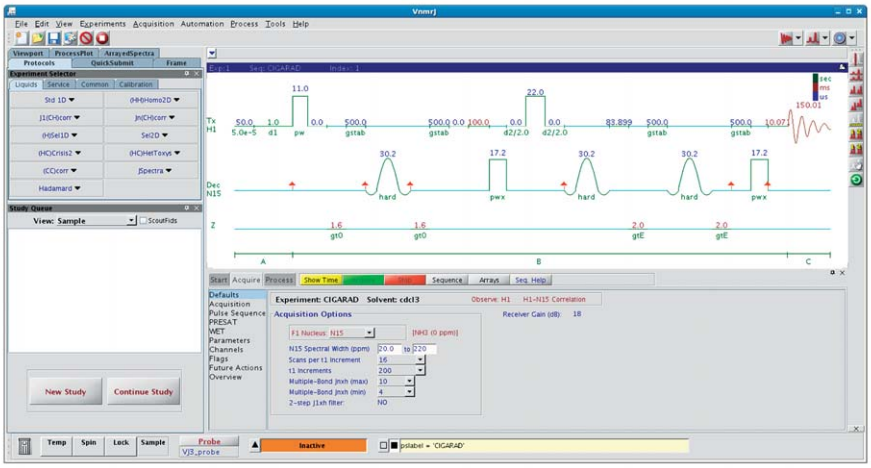

Figure 5. Setting up the CIGAR pulse sequence, step 3.

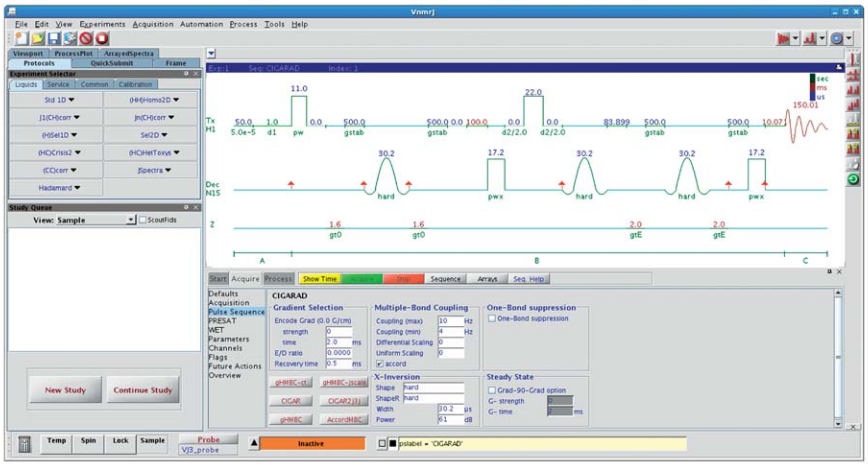

Figure 6. Setting up the CIGAR pulse sequence, step 4.

2. Select **N15** for the F1 Nucleus (see Figure 4).

3. Set the parameters for **spectral width**, **number of transients**, and the values for the **minimum** and **maximum coupling constants** to be sampled. Optimal experimental conditions depend on the sample being investigated, but for typical survey spectra values of 16 transients, 200 increments, and a coupling constant range of 4-10 Hz results in an experiment time of about 2.5 hours and should provide suitable data on samples in the 50-75 mM range. Be careful when selecting the spectral window as there are two common reference scales in use for ^{15}N, based on ammonia or nitromethane (see Figure 5). (The reference standard can be changed using **Tools→Select Reference Standard**.)

4. The sequence can be shortened by allowing one-bond ^{1}H-^{15}N responses to also be observed in the final data set. This is accomplished by deselecting the **one-bond suppression** checkbox on the **Pulse Sequence** panel of the **Acquire** tab (see Figure 6). This will allow both one-bond and long-range responses to ^{15}N to be collected in one data set and, as the number of nitrogen responses in a typical molecule are few, is usually not an issue for interpretation. The one-bond correlations will appear as a box of 4 responses centered on the true correlation frequencies. Aside from the coupling constant range, additional parameters on this panel typically do not need to be adjusted. **Note**: there are buttons on this panel to quickly switch to other types of HMBC experiment not described in this application note.

5. If it is anticipated that this experiment will be used on a routine basis, the **Study Clone** utility can be used at this point to save the current experimental set up in the Experiment Selector (See Chapter 3 of the Automation Guide).

Conclusions

The CIGAR experiment can provide very useful structural information on a wide variety of nitrogen-containing compounds. It is easy to set up and use in VnmrJ 3, allowing all levels of spectroscopists to benefit from this technique.

References

1. Berger, S., and Wagner, R., ACCORD-HMBC: a superior technique for structural elucidation. *Magn. Reson. Chem.*, **1998**, 36:(S1)S42-S44.
2. Hadden, C. E., Martin, G. E., and Krishnamurthy, V. V. Constant time inverse-detection gradient accordion rescaled heteronuclear multiple bond correlation spectroscopy: CIGAR-HMBC. *Magn. Reson. Chem.,* **2000**, 38:143-147.
3. Kline, M., and Cheatham, S. A robust method for determining ^{1}H-^{15}N long-range correlations: ^{15}N optimized CIGAR-HMBC experiments. *Magn. Reson. Chem.,* **2003**, 41:307-314.

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